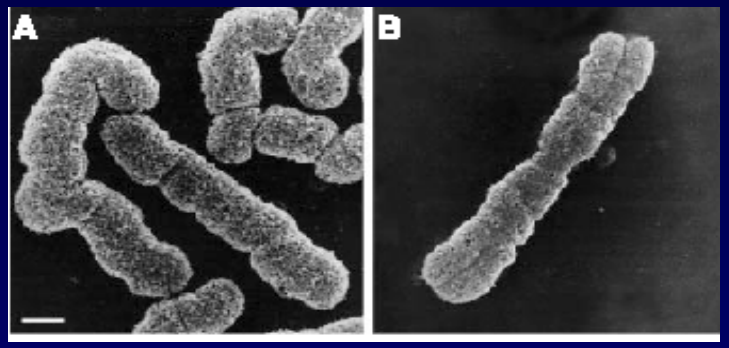
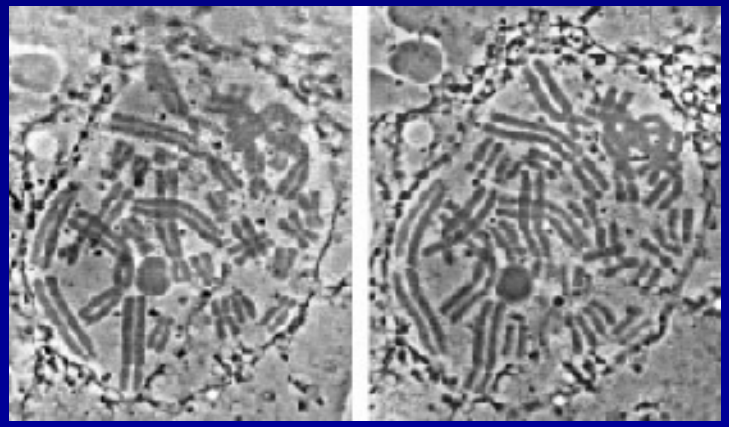


Cytogenetics

Dr. Saba Abdi



Definitions

Cytogenetics

Visual study of chromosomes **, their structure and their inheritance at microscopic level.**

Karyotype

Each species has a characteristic number of chromosomes and this is known as *karyotype. Eg.* Bacteria =1,

Fruit fly =8, Rabbit=44, Human=46

Idiogram

Drawing of photograph of karyotype of a perticular cell

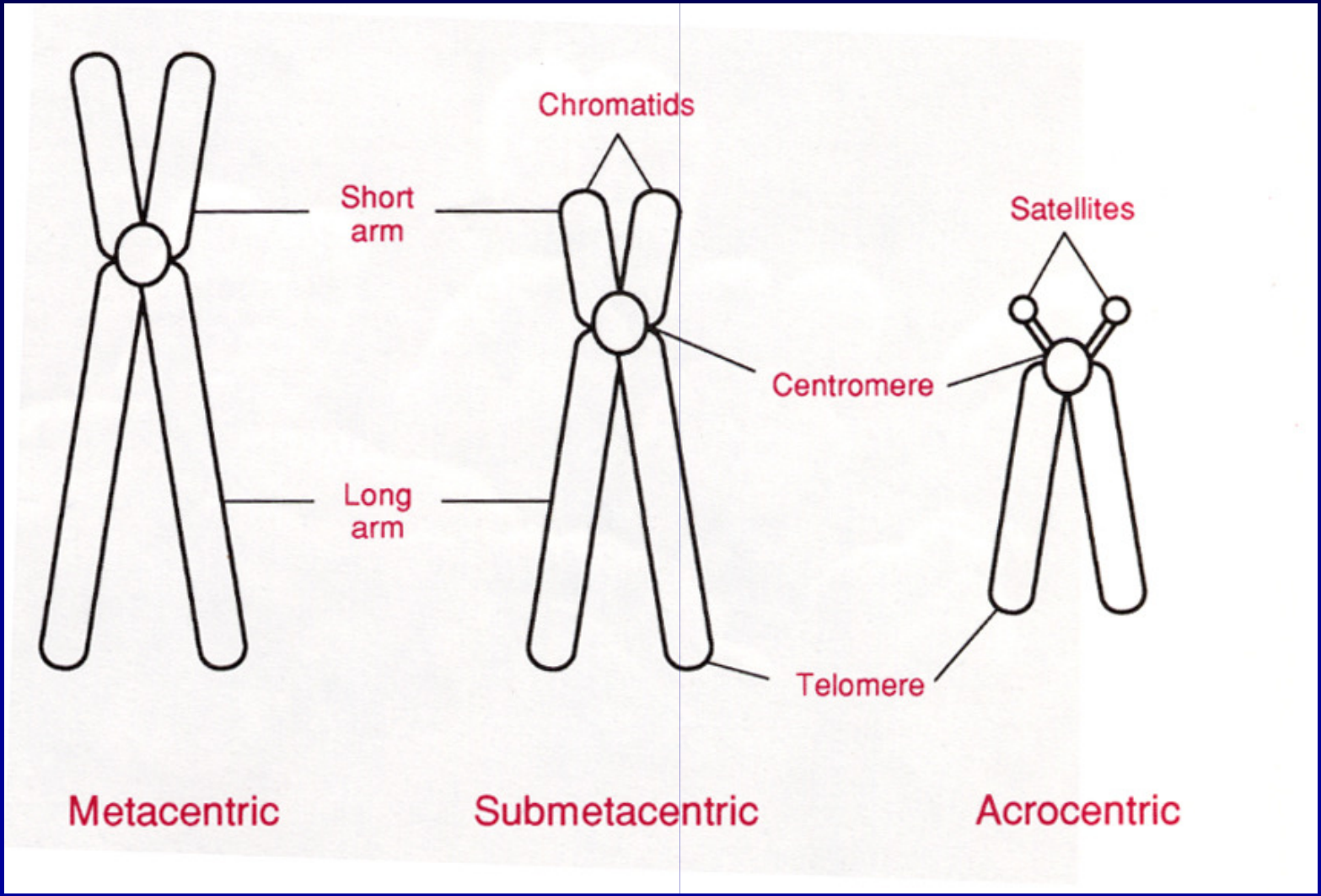
Chromosomes

- Centromere – helps in chromosomal movement during cell division
  - divides the chromosomes into short (p) and long (q) arms
- Telomere - tip of each chromosome
  - seal chromosomes and retain chromosome integrity
  - telomere consists of tandem repeats TTAAGGG
  - maintained by enzyme - telomerase
  - reduction in telomerase and decrease in number repeats important in ageing and cell death

Chromosomes

- Classified according to position of centromere
- Central centromere - metacentric
- Sub-terminal centromere - acrocentric
  - have satellites which contain multiple copies of genes for ribosomal RNA
- Intermediate centromere - submetacentric

Chromosomes



Chromosomes

- 22 autosomes and sex chromosomes in pairs
- Classified according to:
  - Length
  - position of centromere
  - presence or absence of satellites
- Chromosomes divided into groups labelled A-G

  - A 1-3
  - B 4-5
  - C 6-12 + X
  - D 13-15
  - E 16-18
  - F 19-20
  - G 21-22 +Y

Karyotyping

- Staining methods to identify chromosomes

- G banding - Giemsa
- Q banding - Quinacrine
- R banding - Reverse
- C banding - Centromeric (heterochromatin)

- Ag-NOR stain - Nucleolar Organizing Regions (active)

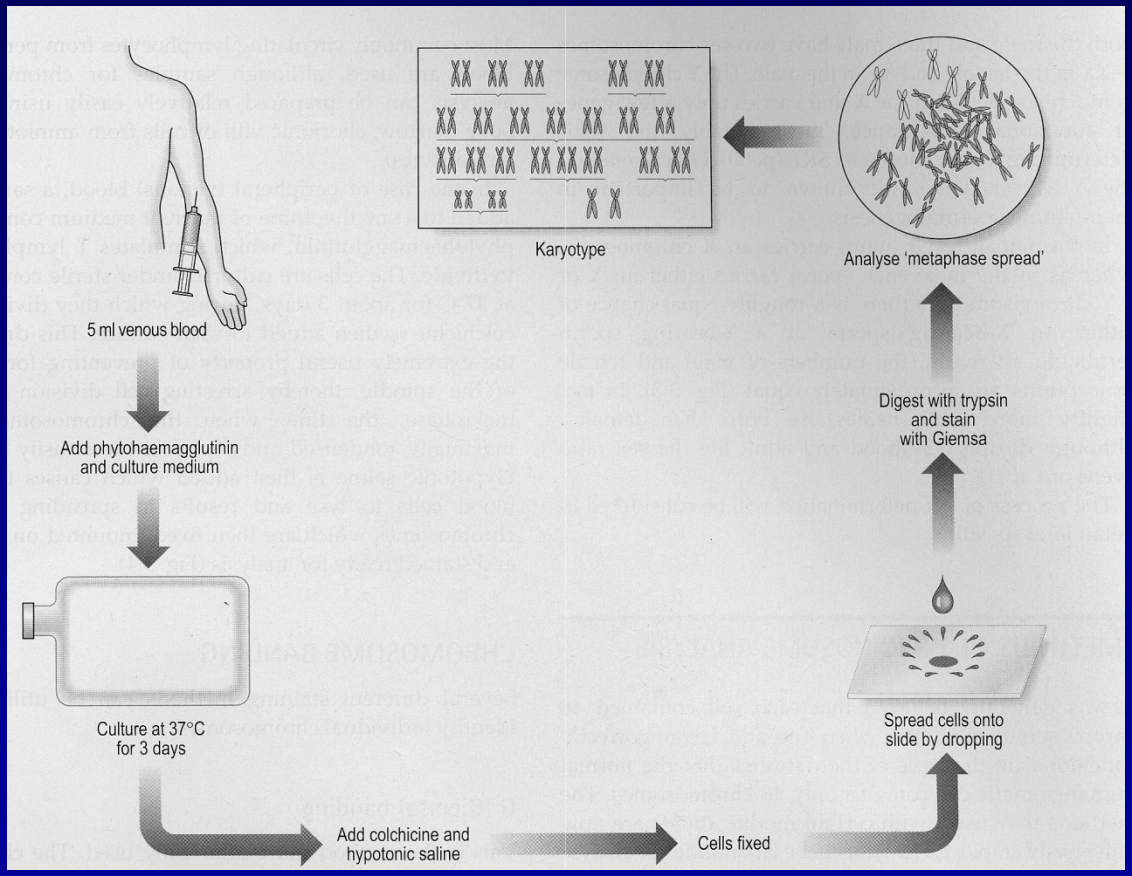
Karyotyping – cell preparation

- Need metaphases
- Culture cells until sufficient mitotic activity
- Add colchicine (or colcemid) to arrest in metaphase
  - prevents mitotic spindle fibres forming
- Add hypotonic salt solution to swell cells
- Fix with mix of methanol;acetic acid
- Want long chromosomes with none overlapping

G banding

- Most common method used
- Chromosomes treated with trypsin
  - denatures protein
- Giemsa stain
  - each chromosome characteristic light and dark bands
  - 400 bands per haploid genome
  - Each band corresponds to 5-10 megabases
  - High resolution (800 bands ; prometaphase chromosome)
    - use methotrexate and colchicine
- Dark bands are gene poor

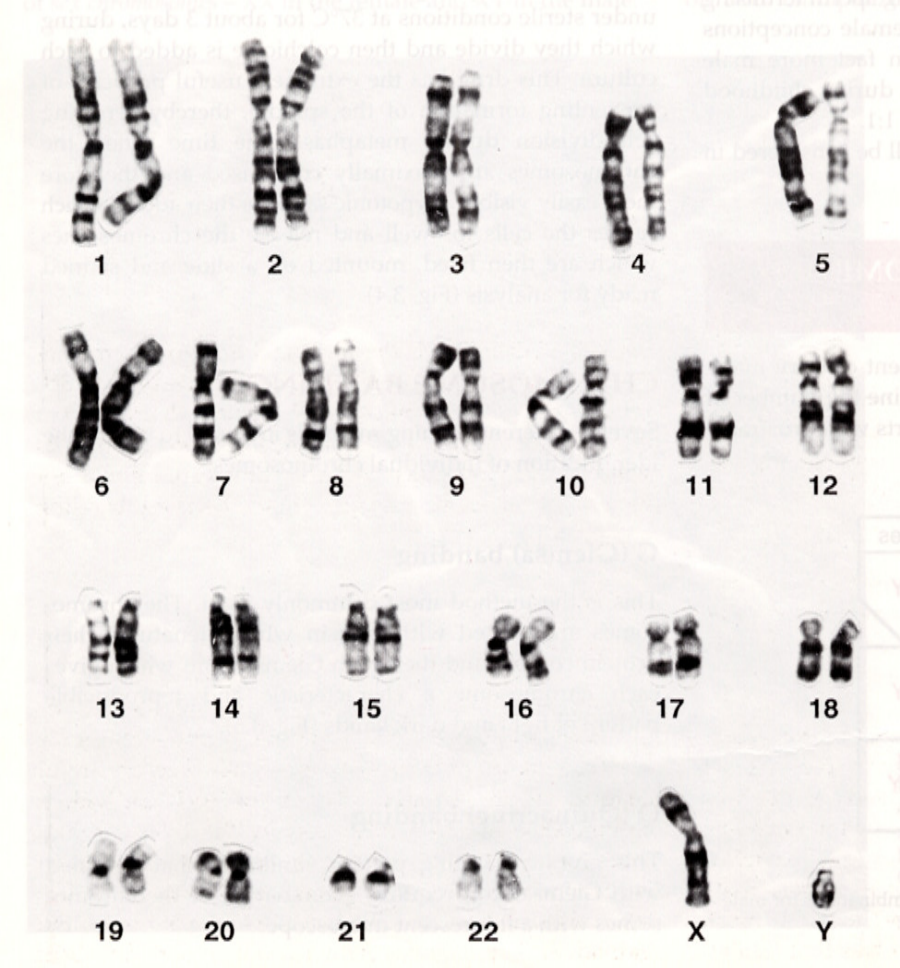
Preparation of G banded karyotype



G banding

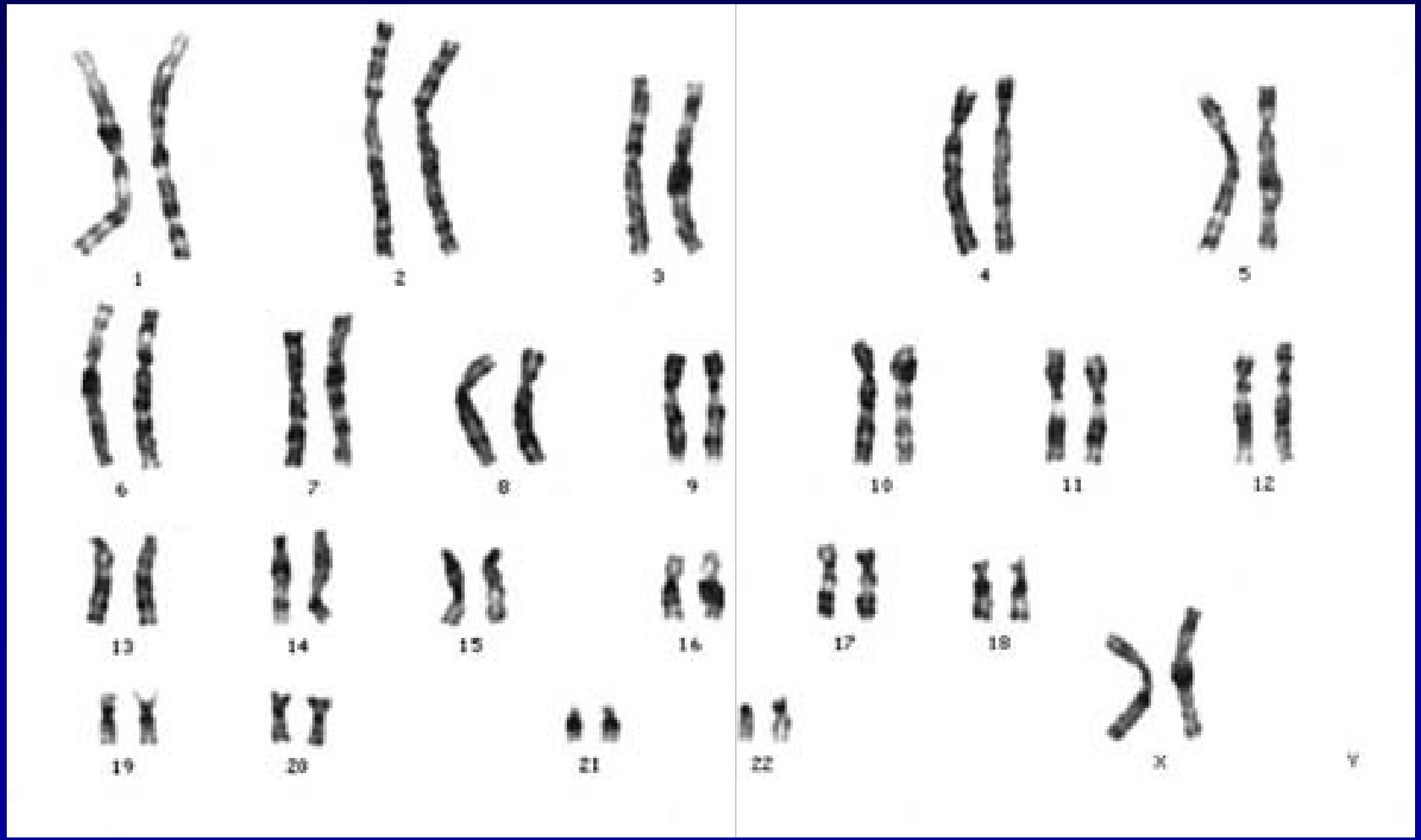
- Metaphase spreads
- Count chromosomes in 10-15 metaphases
- If mosaicism suspected, count 30
- Detailed analysis of 3-5 metaphases
- Used to photograph and cut out
- Now computer programmes

Normal male karyotype



- 13, 18, 21 gene poor
  - Very dark chromosomes
- 21 smaller than 22
  - Wrong way around
- 22 twice as many genes as 21
  - 200 on 21
  - 400 on 22

Normal female karyotype

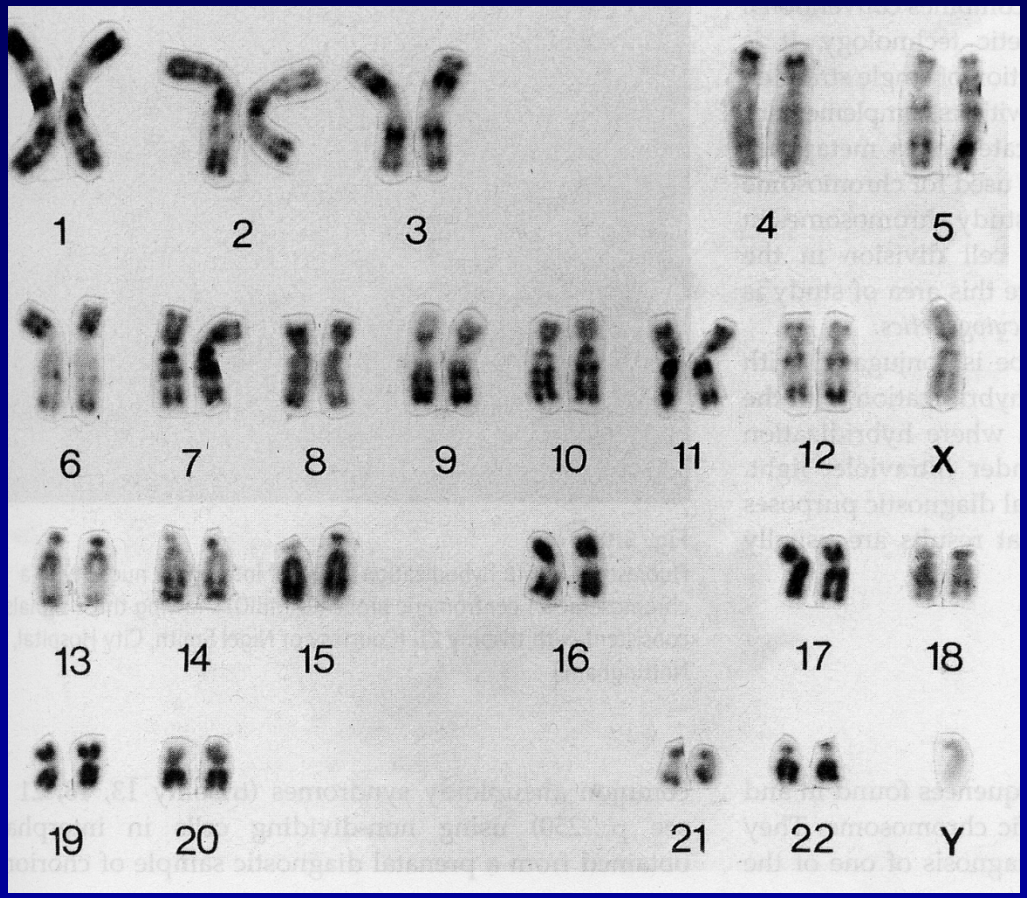


Q banding

- Used especially for Y chromosome abnormalities or mosaicism
- Similar pattern to G banding
  - But can detect polymorphisms
- Needs fluorescent microscope

R banding

- Used to identify X chromosome abnormalities
- Heat chromosomes before staining with Giemsa
- Light and dark bands are reversed

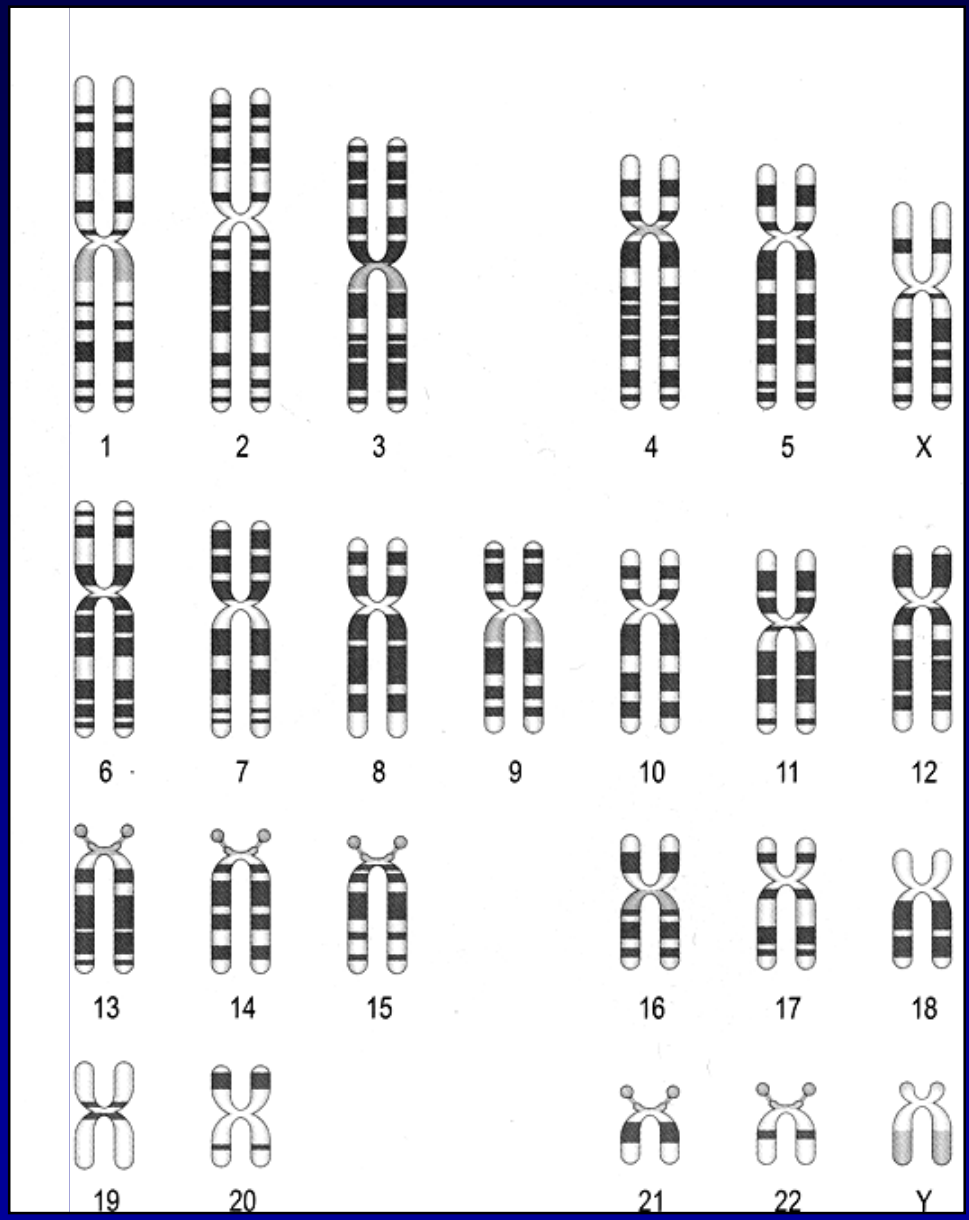


C banding

- Used to identify centromeres / heterochromatin
- Heterochromatic regions
  - contain repetitive sequences
  - highly condensed chromatin fibres

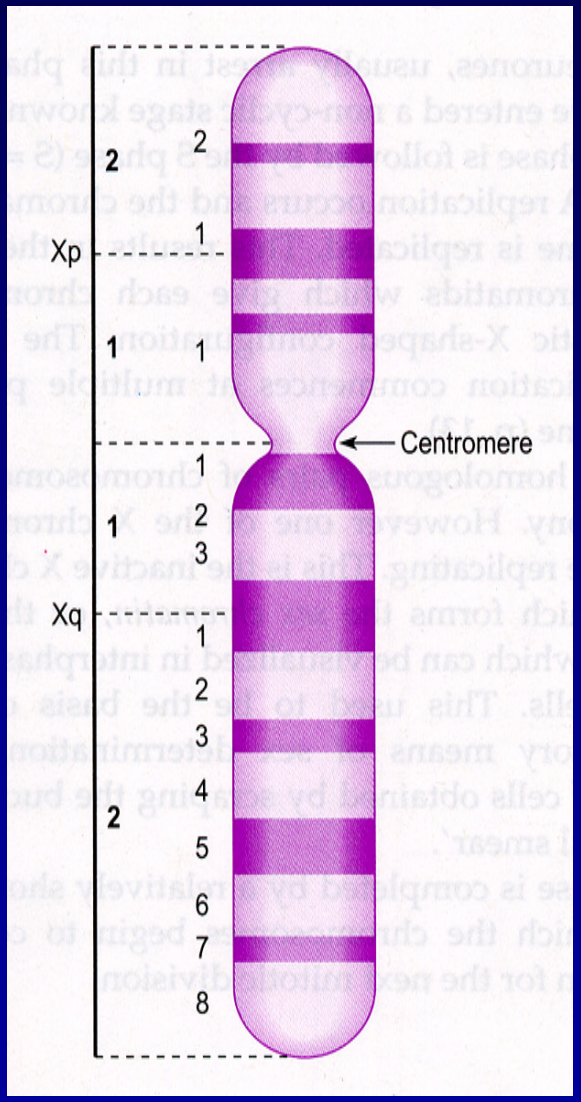
- Treat with chromosomes with
  1. Acid
  2. Alkali
  3. Then G band

Idiogram



ISCN

- International System for Human Cytogenetic Nomenclature
- Each area of chromosome given number
- Lowest number closest (proximal) to centromere
- Highest number at tips (distal) to centromere



ISCN

- del - deletion
- dic - dicentric
- fra - fragile site
- i - isochromosome
- inv - inversion
- p - short arm
- r - ring
- der - derivative
- dup - duplication
- h - heterochromatin
- ins - insertion
- mat - maternal origin
- q - long arm
- t - translocation

ISCN

,

46,XX,del(5p)

- separates
  - chromosome numbers
  - sex chromosomes
  - chromosome abnormalities

;

46,XX,t(2;4)(q21;q21)

- separates
  - altered chromosomes
  - break points in structural rearrangements involving more than 1 chromosome

ISCN

- Normal male
  - 46,XY
- Normal female
  - 46,XX

Types of chromosome abnormalities

- Numerical
  - Aneuploidy (monosomy, trisomy, tetrasomy)
  - Polyploidy (triploidy, tetraploidy)
- Structural
  - Translocations
  - Inversions
  - Insertions
  - Deletions
  - Rings
  - Isochromosomes
  - ESAC

Numerical

- Aneuploidy
  - Autosomal trisomy, 47
  - Sex chromosomes, 45, 47, 48, 49
- Polyploidy
  - Whole chromosome set
  - Triploidy, 69
  - Tetraploidy, 92

Aneuploidy

- Almost all been found in oocytes and early embryos, trisomies and monosomies
- Most lethal (miscarry)
- Do not see in pregnancy or live born
- Exceptions sex chromosomes and Down
- Some aneuploidy is age related

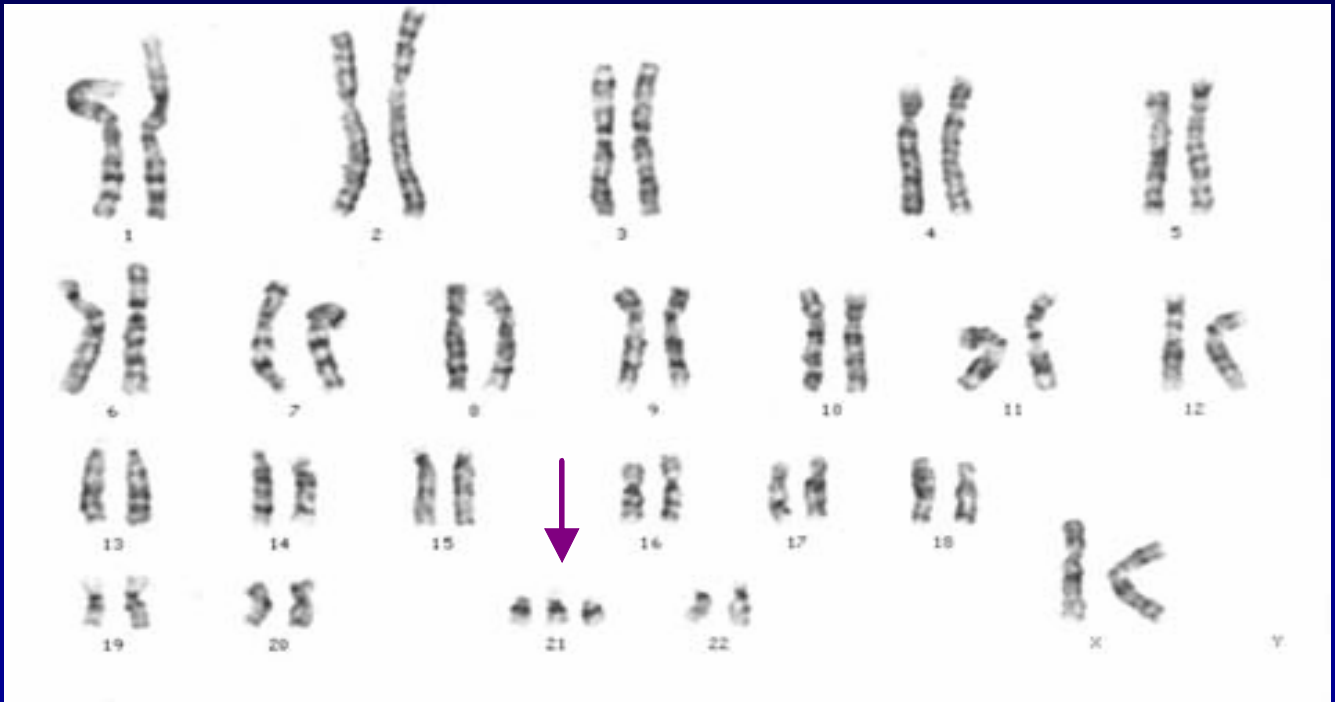
Sex chromosomes

- Abnormalities more tolerated
- If have extra Y, few genes mainly for sex determination
- If have extra X, excess X is inactivated
- Monosomy X, Turners
  - Majority die during development
  - Only small proportion survive to birth
  - Short and infertile

Sex chromosome abnormalities

- Turner Syndrome 45,XO (female)
- Trisomy X 47, XXX (female)
- Klinefelter Syndrome 47,XXY (male)
- Extra “Y” chromosome 47,XYY (male)

Down syndrome, trisomy 21



47,XX,+21 or 47,XY,+21

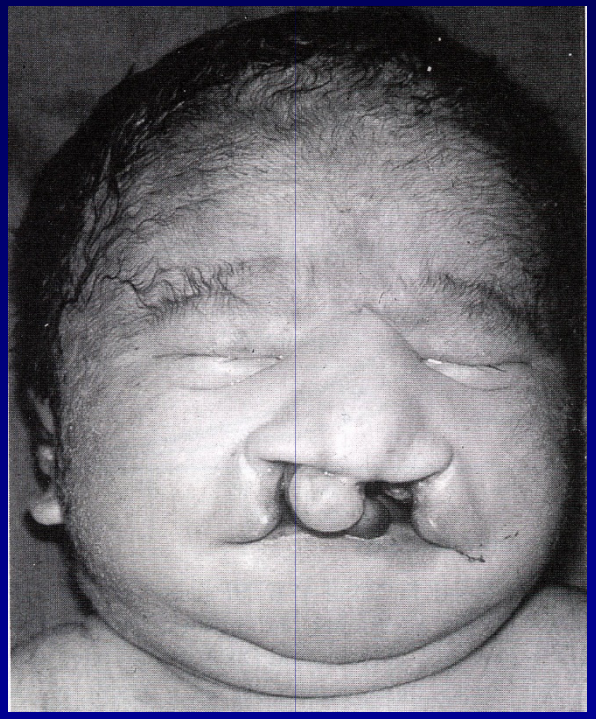
Incidence at birth 1/700

Edwards syndrome, trisomy 18

47,XX,+18 or 47,XY,+18

Incidence at birth 1/5,000

Patau syndrome, trisomy 13



47,XX,+13 or 47,XY,+13

Incidence at birth 1/5,000

Structural

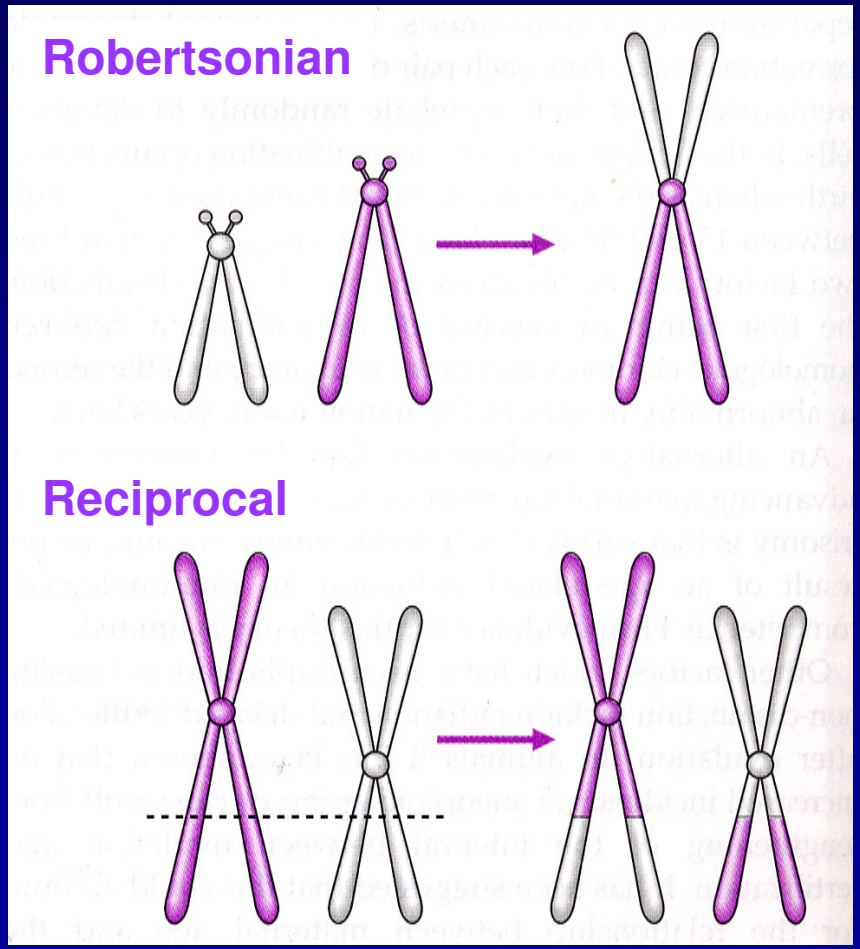
- Breakage in at least 1 chromosome
- Translocations
  - 2 different chromosomes break and rejoin incorrectly
- Inversions
  - 2 breaks in same chromosome
- Insertions
  - Piece of chromosome inserted
- Deletions
  - Piece of chromosome missing

Chromosome breaks

- Once chromosome broken by some means
- Unstable situation as telomeres not at end
- Usually join up to other piece

Translocations

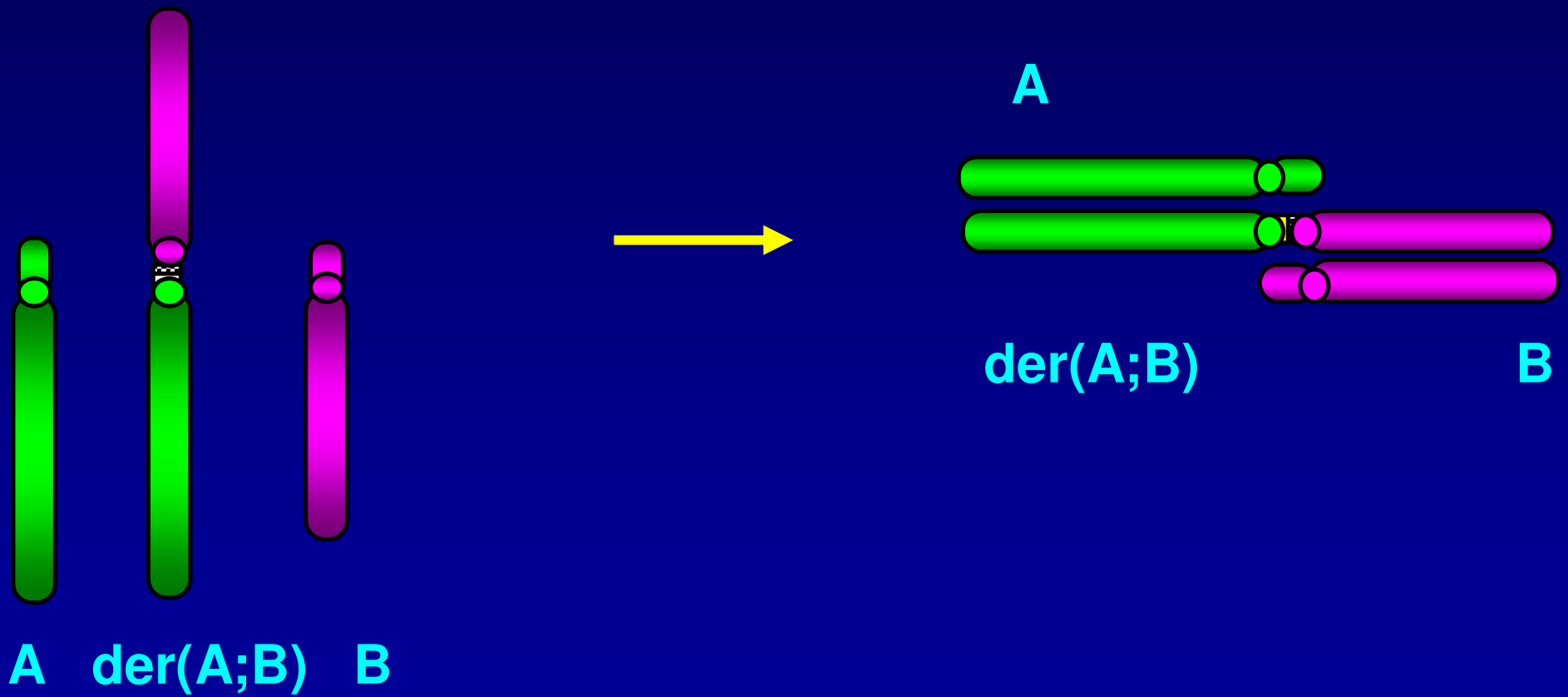
- Chromosome moves from normal position to abnormal position
- Robertsonian
  - Acrocentric chromosomes
  - D and G groups (13, 14, 15, 21, 22)
- Reciprocal
  - Any chromosome



Robertsonian translocations

- Lose satellite and short arms
  - Genes for rRNA
  - Repeated on other acrocentric chromosomes
- Reduce chromosome number by one (45)
  - but no loss of chromatin from long arms
- Phenotypically normal – problems at meiosis
- Involved in evolution

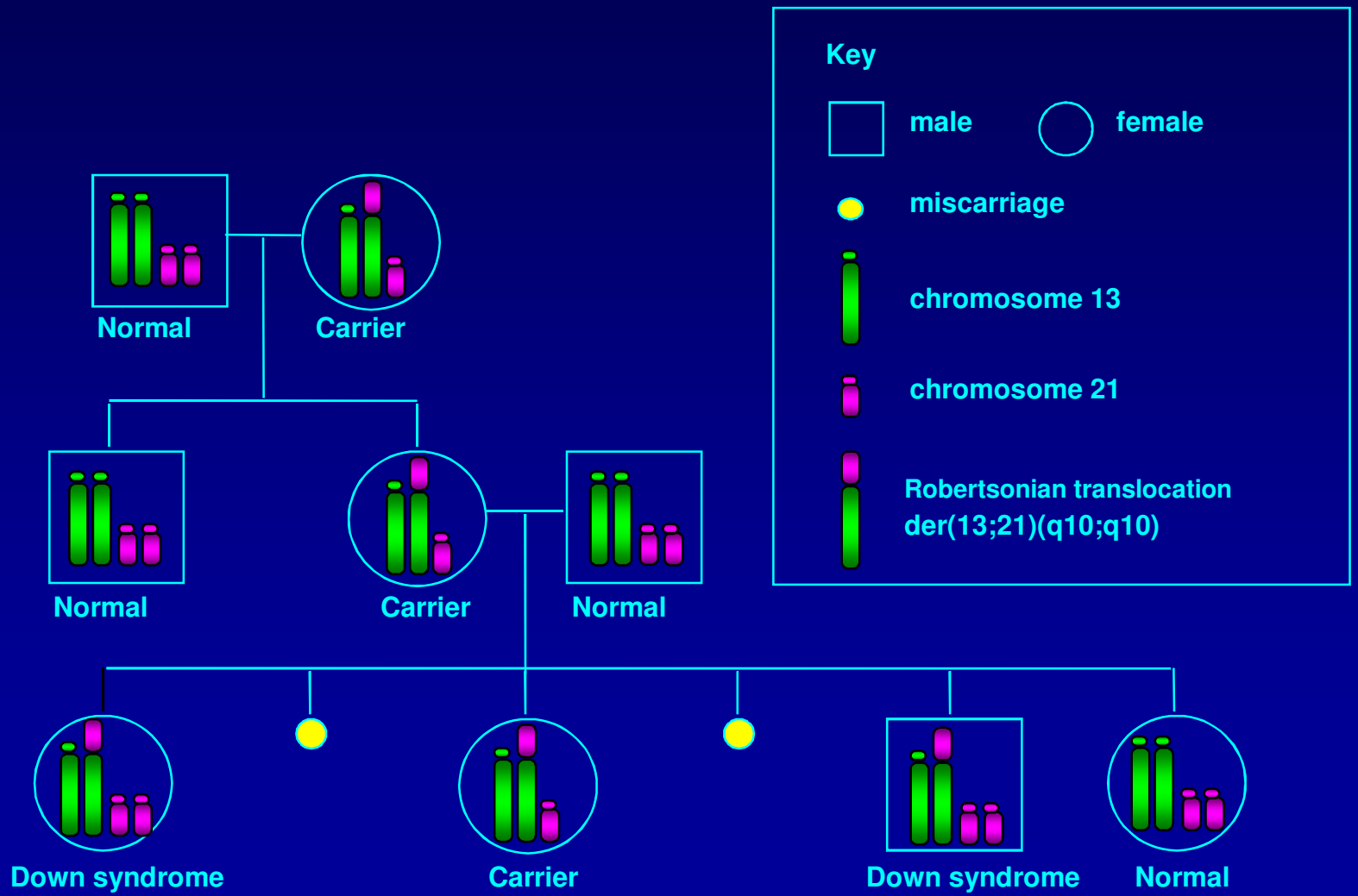
Robertsonian translocations



Robertsonian translocations

- D:G translocation
  - Often 14:21 joined
- G:G translocation
  - 21:22 joined
  - 21:21 joined
    - 21 smallest chromosome

Robertsonian translocation family pedigree

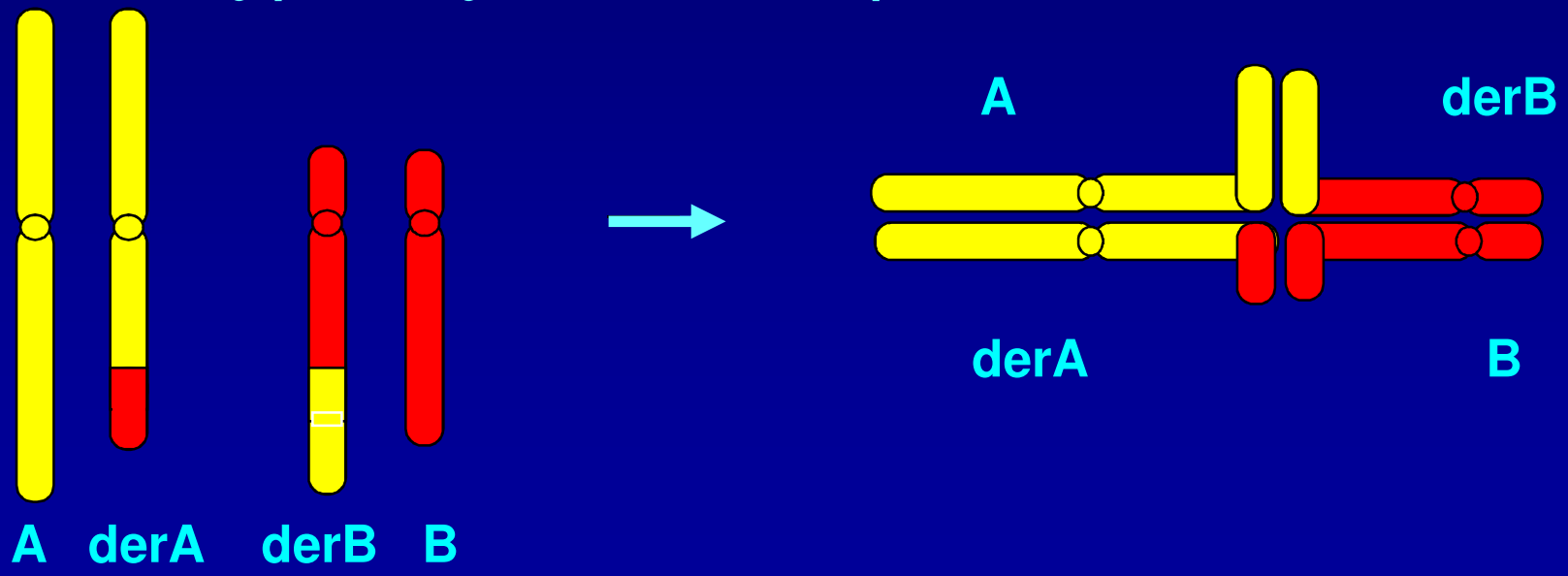


Robertsonian translocations

- 45,XY,der(13q;14q)(q10;q10)
- 45,XX,der(13q;21q)(q10;q10)

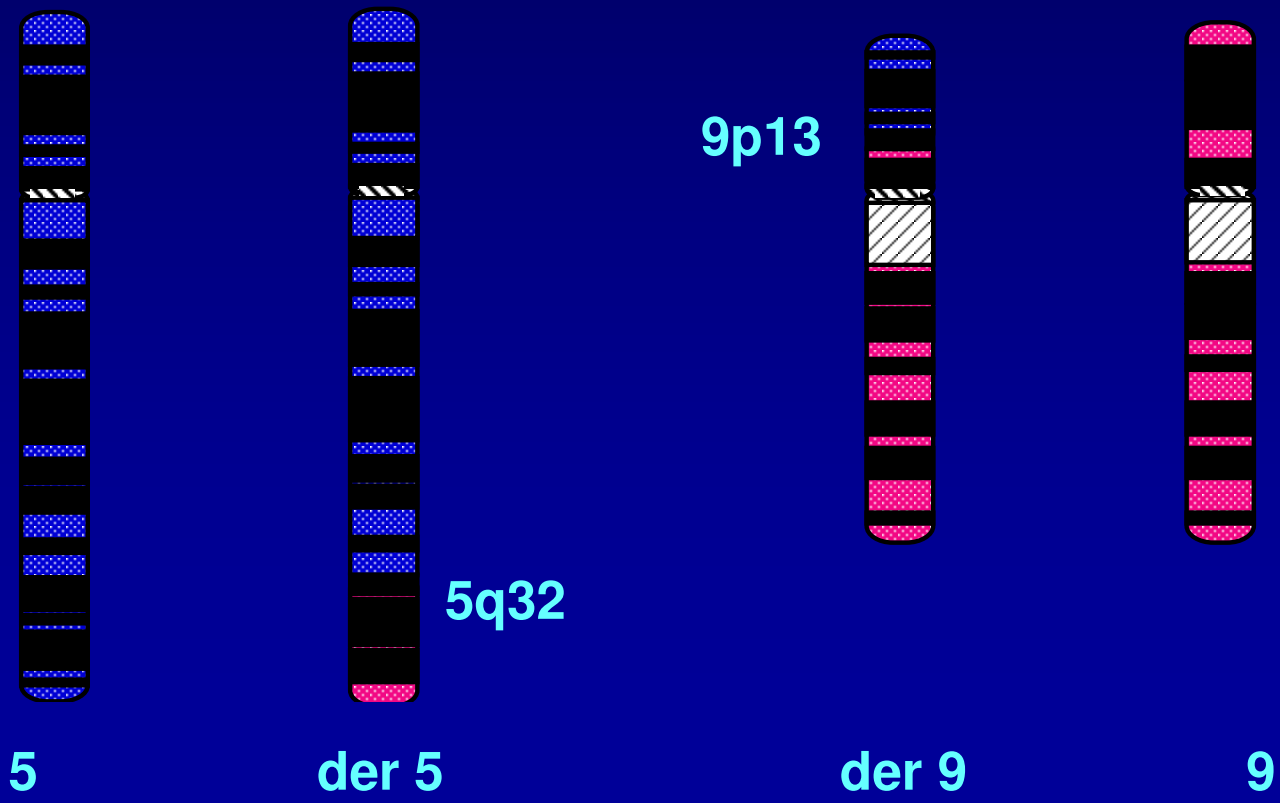
Reciprocal translocations

- More common than Robertsonian
- Break in any chromosome at any point
- Phenotypically normal – problems at meiosis



Reciprocal translocation

46,XX,t(5;9)(q32;p13)

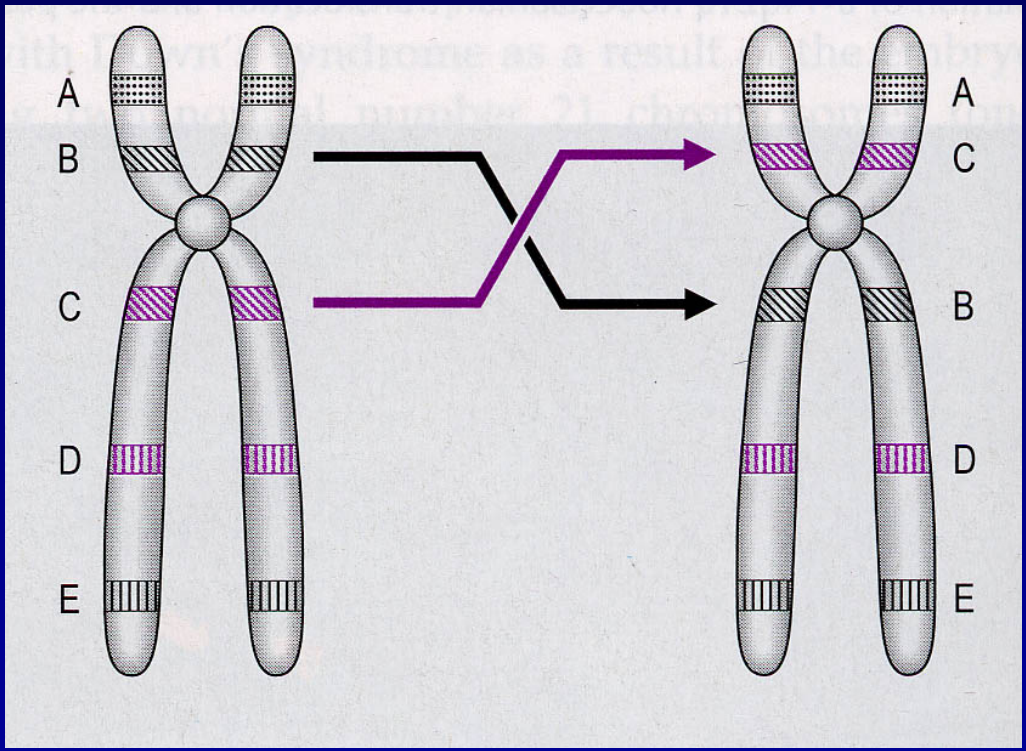


Inversions

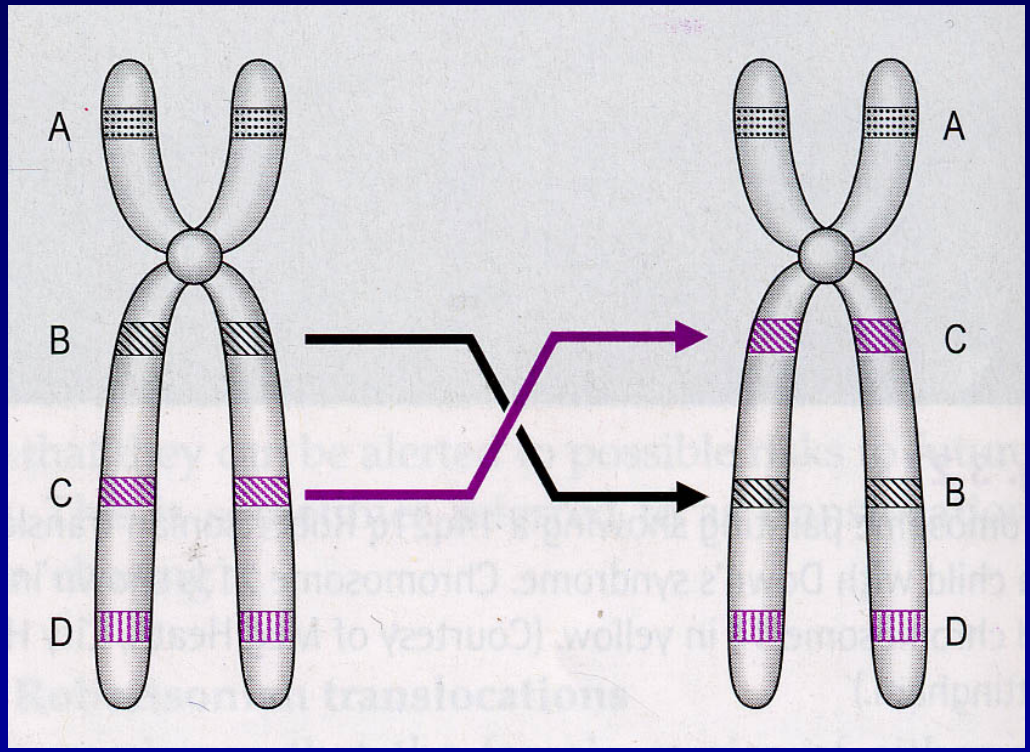
- Reversal of segment of chromosome
  - If too small cannot detect by karyotype
  - Very rare in humans
  - Selected against as would get reduced fertility
- Pericentric
  - reversed segment includes centromere
- Paracentric
  - within one chromosome arm

- Paracentric inversion
  - main difference in karyotypes of great apes and humans so important in evolution

Inversions



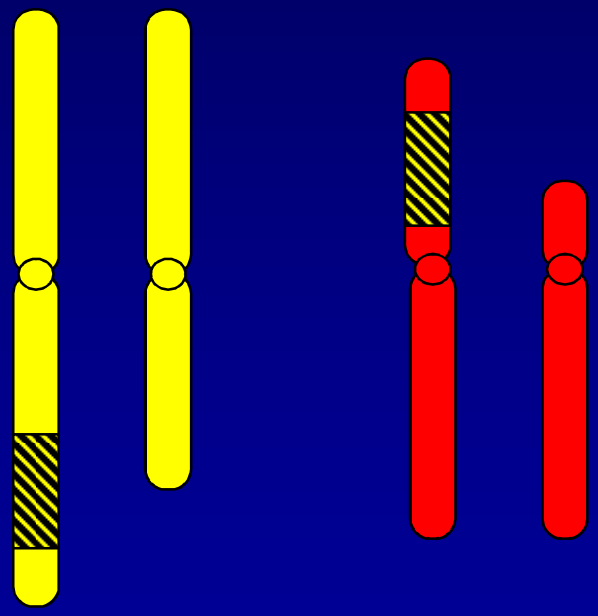
Pericentric



Paracentric

Insertions

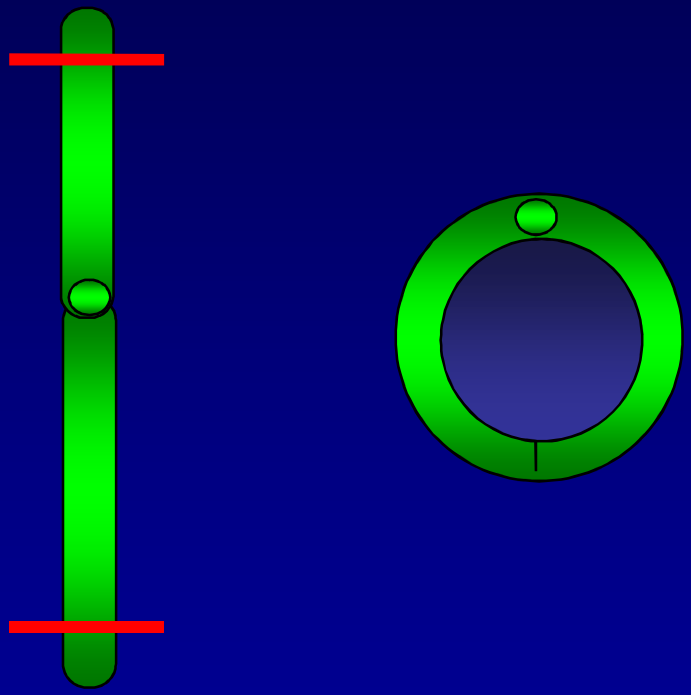
- Segment of 1 chromosome inserted into another



Deletions

- Terminal
  - loss of end of chromosome
  - 46,XY,del(10)(q26) missing long arm of 10
- Interstitial
  - loss of segment from within chromosome
  - 46,XY,del(10)(q24q26) missing segment of 10
- All result in unbalanced karyotype
- Partial monosomy
- Serious clinical effect

Ring chromosome

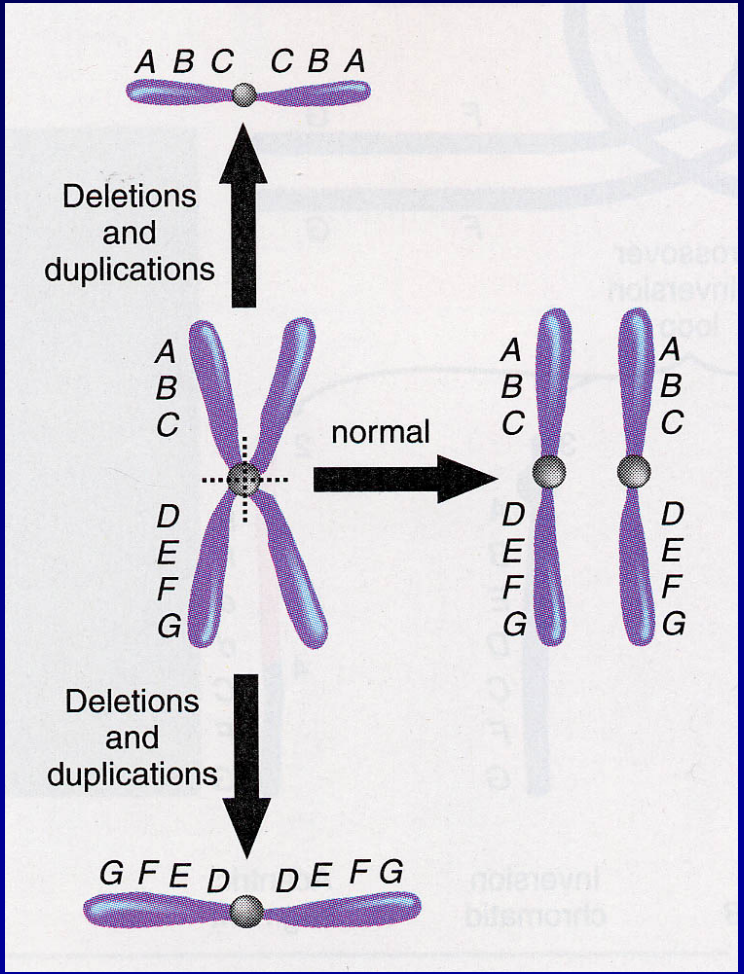


A

derA

Isochromosome

- Two copies of the same arm
- Mirror image around centromere
- Centromeres part in wrong plane
  - Monosomy for 1 chromosome arm
  - Trisomy for the other arm



ESAC

- Extra Structurally Abnormal Chromosome
- Abnormal chromosome in addition to 46
- Small and difficult to identify
- Sometimes called marker chromosomes
- Difficult to work out effect on person
- May be benign or cause serious mental handicap